THE DISTRIBUTION OF X-RAY INDUCED CROSSOVERS FROM CURLY INVERSION HETEROZYGOTES OF DROSOPHILA MELANOGASTER FEMALES

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Introduction.—That crossing over may occur in oögonial cells was suggested by the results of experiments by Whittinghill[1] in which crossing over was induced by x-rays in the *X*-chromosomes of Drosophila females homozygous for the *c3G* asynaptic factor. It had previously been demonstrated that induced crossovers recovered from Drosophila males were of spermatogonial origin.[2, 3] Cooper's observations[4] of chiasmata in gonial cells of both sexes of Drosophila may provide cytological foundation for the hypothesis of gonial origin of some crossovers, particularly those which are x-ray induced. Somatic crossing over, the basis for the occurrence of twin spots in the hypodermis of Drosophila males and females,[5] closely parallels gonial crossing over in the formation of daughter cells which have become homozygous distally. The possible consequences of oögonial crossing over upon linkage data, as recently discussed by Whittinghill,[6] illustrate the need for further investigation of this phenomenon. This paper reports the results of x-ray induced crossing over in Curly inversion heterozygotes of *Drosophila melanogaster* females, and these results are interpreted in relation to normal, or random, meiotic events as opposed to oögonial events of recombination.

Experimental Methods.—Stocks and experimental procedures, with the exception of the treatment employed in this work, were identical with those described in the companion paper[7] in these PROCEEDINGS. Twenty-eight adult virgin females between six and twelve hours old were placed in a 0.7-ml. gelatin capsule and exposed to a total x-ray dose of 2250 r, which was received at the rate of 350 r per minute from a distance of 20 cm. through a 4-mm. aluminum filter from a tube operating at 140 kv. These females were allowed to feed for one day before being testcrossed separately to 4 or 5 *lt/lt* males in consecutive half-pint bottles for life. Transfers, without etherization, were made every other day for the first seven cultures and at longer intervals for the final three cultures. As in the former experiment, all cultures were coded so that classifications were performed without bias. Uncertain phenotypes were determined by testcrosses to light homozygotes. Although the spontaneous recombination data of the preceding report have been employed to some extent as controls, accurate comparison of the frequency of crossing over is not possible because of the absence of randomly determined recombination offspring.

The authors have concentrated their study on the actual distribution of induced recombinants in an attempt to detect the place and manner of their origin.

Results.—Although fertility of the irradiated adults was decidedly lower than that of controls, recombination values were greatly increased throughout the egg-laying period (table 1). This persistence of the increase in recombination for as long as 31 days after treatment may have been the result of early as well as late oögonial cells having been affected. Any influence of age on spontaneous recombination is obscured by the much larger effect of the x-rays. This effect included a real peak in recombination between 4 and 8 days after treatment, as shown by the standard errors for cultures 3 and 4 in table 1.

TABLE 1

COMPARATIVE EFFECTS OF AGE (*C*) AND X-RAYS (*X*) ON RECOMBINATION VALUES IN CONSECUTIVE TESTCROSS CULTURES OF *S Pfd/Cy lt L*^{4} FEMALES

CULTURE NUMBER	FERTILE FEMALES (*C*)	FERTILE FEMALES (*X*)	TOTAL PROGENY (*C*)	TOTAL PROGENY (*X*)	PER CENT RECOMBINATION (*C*)	PER CENT RECOMBINATION (*X*)
1	18	20	1334	60	0.82 ± 0.24	5.00 ± 2.81
2	18	21	1681	532	0.83 ± 0.22	3.95 ± 0.27
3	18	16	1414	250	0.14 ± 0.10	10.80 ± 1.96
4	18	14	1511	549	0.07	7.47 ± 1.12
5	18	13	1594	379	0.06	5.01 ± 1.12
6		13		631		3.65 ± 0.75
7		12		467		1.93 ± 0.64
8		9		390		2.82 ± 0.84
9		6		427		4.22 ± 0.97
10		1		34		11.76 ± 5.52
Totals			7534	3719	0.38 ± 0.07	4.73 ± 0.35

Three results which are unexpected in random meiotic processes are revealed when the data are recombined into familial groups (table 2). The first notable feature of the table consists of large deviations from the mean total recombination value of 4.73 ± 0.35 per cent. These values range from zero to above 12 per cent. Secondly, the usual assumption of linearly random exchanges is not substantiated due to the wide variations in proportions of region 2 to region 3 crossovers as exhibited chiefly in the families of Females 5, 11 and 13, as compared with 16. Thirdly, pronounced inequalities in complementary crossover classes within a region may be observed in the crossover columns of Females 5, 11, 16 and 23. All of these three forms of variation from female to female have been shown to be expected consequences of oögonial crossing over[1, 6] and may be interdependent to some extent. The most diagnostic of these consequences, and the one which lends itself most readily to analysis, is the extent of imbalance between complementary crossover classes.

TABLE 2

FAMILIAL DISTRIBUTION OF TESTCROSS OFFSPRING FROM IRRADIATED ADULT *S Pfd/Cy lt L*^{4} FEMALES

FEMALE NUMBER	NON-CROSSOVERS		SINGLE CROSSOVERS				TOTAL PROGENY	PER CENT RECOMBINATION
	S Pfd	*Cy lt L*^{4}	REGION 2		REGION 3			
1	96	98	3	0	2	2	201	3.48 ± 1.29
2	128	146	5	2	5	2	288	4.86 ± 1.27
4	86	95	2	3	3	0	189	4.23 ± 1.46
5	274	261	13	7	0	0	556[a]	3.96[a] ± 0.83
8	120	135	1	1	1	0	258	1.16 ± 0.67
11	103	85	8	2	0	1	199	5.53 ± 1.62
12	33	25	3	3	1	1	66	12.12 ± 4.03
13	135	136	9	10	4	2	296	8.45 ± 1.62
15	7	8	0	0	1	0	16	6.25 ± 6.06
16	225	233	1	1	12	5	477	3.98 ± 0.90
18	238	230	5	7	6	5	491	4.68 ± 0.95
19	62	59	4	2	2	0	129	6.20 ± 2.12
20	36	49	0	1	1	0	87	2.30 ± 1.67
22	21	33	3	1	1	3	62	12.90 ± 4.26
23	58	62	0	2	5	0	127	5.51 ± 2.02
26	85	91	5	2	2	1	186	5.38 ± 1.65
12 others	39	52	0	0	0	0	91	0
Totals	1746	1798	62	44	46	22	3719	4.73 ± 0.35

[a] Includes 1 *S lt Pfd* double crossover.

TABLE 3

CHI SQUARE ANALYSIS OF THE DISTRIBUTION OF COMPLEMENTARY CROSSOVERS WITHIN FAMILIES OF IRRADIATED FEMALES

FEMALE NUMBER	REGION 2			REGION 3		
	S lt L^4: *Cy Pfd*	*p*	$-\log_e$	*S L*^{4}: *Cy lt Pfd*	*p*	$-\log_e$
1	3:0	0.125	2.080	2:2	0.500	0.693
2	5:2	0.227	1.483	5:2	0.227	1.483
4	2:3	0.500	0.693	3:0	0.125	2.080
5	13:7	0.132	2.025	(0:0)	...	....
8	1:1	0.500	0.693	(1:0)	...	....
11	8:2	0.055	2.904	(0:1)	...	....
12	3:3	0.500	0.693	1:1	0.500	0.693
13	9:10	0.500	0.693	4:2	0.344	1.067
16	1:1	0.500	0.693	12:5	0.072	2.632
18	5:7	0.387	0.950	6:5	0.500	0.693
19	4:2	0.344	1.067	2:0	0.250	1.386
22	3:1	0.313	1.161	1:3	0.313	1.161
23	0:2	0.250	1.386	5:0	0.031	3.478
26	5:2	0.227	1.483	2:1	0.500	0.693
Total $-\log_e$			18.004			16.059
Chi-square	(28 d. f.)		36.008	(22 d. f.)		32.118
Probability			0.154			0.079

Joint Probability = 0.0121

In order to answer the question whether the observed deviations from equality are greater than those allowed by chance, the following analysis was performed. Probabilities of obtaining the observed distribution of complementary crossover classes in each family or of any distribution deviating more in the same direction were computed (table 3). The $-\log_e$, equal to $chi^2/2$ associated with two degrees of freedom, is additive in character,[8] and this allows an over-all probability to be obtained for either region for all families. Since the two regions concerned are on opposite sides of the centromere, one may assume that crossings over occurring simultaneously in these regions are independent of each other. Distributions of 1:0 or 0:1 have been omitted from calculations, for although they add two degrees of freedom to the total, they can contribute no useful information here.

While there are no individual probabilities in the above table which are significant alone, there appears to be a definite excess of distributions of low likelihood as shown by the probabilities of 0.154 and 0.079 for regions 2 and 3, respectively, all families combined. Furthermore, the joint probability for both regions is 0.0121, so that a similar over-all distribution may be expected to occur by chance alone only one time in one hundred like experiments.

Viability differences in crossover chromosomes may sometimes account for numerical differences in complementary crossover classes throughout an experiment; however, this possibility may be reasonably eliminated here. Six additional testcrosses of males whose genotypes corresponded to a combination of complementary crossover chromosomes from region 2 exchanges yielded 706 *S lt* L^4 and 792 *Cy Pfd* flies. This inequality is in the opposite direction from that in the crossover progeny from testcrossed females which gave a 62:44 majority to the *S lt* L^4 class. As there is no reason to suspect that the relative viabilities of the two chromosomes may be reversed depending on whether they originate from sperms or eggs, some cause other than viability differences must be responsible for the observed inequalities in complementary crossover offspring from irradiated females. Similar tests yielding 579 SL^4: 530 *Cy lt Pfd* indicate that larval viability differences played little or no part in the 46:22 result from region 3.

Previous studies of induced crossing over in Drosophila have revealed a tendency of some recombinants to appear simultaneously among the testcross progeny, and several instances of this irregularity occurred in this experiment. A cluster of 6 region 2 recombinants distributed equally was recovered from the third culture of Female 13 among a small brood of 19 flies which included only one crossover from the third region. By comparison, the mean brood size per fertile culture was found to be 33.50 of which 1.58 flies would be expected to be recombinants, assuming a ran-

dom distribution. The fourth culture of Female 4 contained only 3 recombinants, identical region 2 triplets, among a total brood of 42. At this same age, Females 11 and 18 each produced identical quadruplets from region 2, and each set was accompanied by twins of the complementary class; in addition, the latter female had twins from region 3. While these examples are from the two cultures which exhibited the maximum recombination values, clustering was not limited to this period. It is very unusual that although Female 5 produced 7 region 2 recombinants out of 182 offspring in Culture 9 (20–26 days), region 3 recombinants were not represented; the Culture 10 (27–31 days) brood of 34 from this same female included 4 crossovers, all from region 2. Other clusters of lesser magnitude might be cited, and it is possible that some clusters were missed due to splitting them when transferring the cluster-producing female into a new culture.

Two light homozygotes, classified with the broods of Females 18 and 23, have not been included in the data because of the possibility of their being contaminants. Yet, they are not too improbable representatives of quadruple crossing over when compared with similar multiple crossovers which appeared in controls. Furthermore, in order to recover any recombinants from a region included in an inversion, at least double crossing over must occur since single exchanges alone lead to the production of lethal dicentrics and acentric chromosome fragments.[9] One is also reminded that although the Curly inversions do severely limit the frequency of single exchanges in adjacent non-inverted regions, the non-random occurrence of recombinants resulting from such exchanges is not explained by the presence of these inversions.

Discussion.—If an exchange of homologous segments of paired bipartite prophase chromosomes should occur in only a relatively few oögonial cells, and the products of such an exchange be erratically reproduced before the initiation of meiosis, one may well expect the resulting recombination offspring to be distributed non-randomly as recorded in this experiment. Or, as suggested by the distribution of spontaneous recombinants from Curly inversion heterozygotes, oögonial *exchange* of homologous segments may not be necessary for agreement with the results if only a localized weakening of one chromatid at this time may predispose meiotic crossing over at this point after several mitotic divisions have intervened. It seems plausible that these alternatives could be tested by a cytological examination of the first meiotic division, for if exchange occurred prior to this division, the regions distal to the break and including the inversion would frequently become homozygous, allowing normal synapsis in this arm and free chiasma formation.

On either hypothesis, it is necessary to assume unequal multiplication of the affected chromosomes, and the agencies responsible for this may be

several. For example, different rates of division might be conditioned by the exchange products themselves, or could be imposed by chance according to the relative positions the daughter oögonia occupy in the ovary; some of them may be crowded aside from the main line of division either to be lost entirely or relegated to later appearance. However, some causal factor other than larval viability differences must operate preferentially in one direction in order to explain the total inequalities in complementary crossover adults. Cell lethals could presumably explain this if some oögonia become homozygous due to prior exchange.

Applying this idea to the present experiment, we may assume that Star and Curly may become homozygous in adjacent cells after region 2 exchanges, and that Pufdi and Lobe can become homozygous after region 3 exchanges. If any of these mutants are homozygous cell lethals (all are homozygous lethal in larvae), they could not cause more than a 50 per cent reduction of any crossover chromosome, since such chromosomes may segregate into heterozygous situations as frequently as into the homozygous condition. We would then infer from the 46 *S* L^4:22 *Cy* lt^3 *Pfd* ratio of recombinants that Pufdi is completely lethal to cells homozygous for this factor; i.e., in *S Pfd/Cy lt Pfd* gonia. In the case of region 2 exchanges, Curly would appear to be detrimental, although not completely lethal, to oögonial cells homozygous for it as judged from the ratio of 62 *S* lt^3 L^4 to 44 *Cy Pfd* recombinants from this region. While this hypothesis is dependent on oögonial exchange, other possibilities may be found which agree equally well with oögonial weakening and subsequent meiotic crossing over.

Two hypotheses of the mode of action of x-rays in inducing crossing over have appeared in the literature, and these may be briefly considered. In attempting to demonstrate a single mechanism of crossing over for both male and female Drosophila, Friesen[10] found that the Curly inversions did not limit x-ray induced crossing over between black and cinnabar in males as much as in females. On the basis of this evidence, the lack of spontaneous male crossing over was ascribed to an incomplete conjugation of homologous chromosomes (the greater the normal degree of synapsis, the greater the depressing influence of the inversions). Friesen further explained the action of x-rays while increasing recombination to result in "a closer intercourse of the long autosomes (first of all in the central regions)" for both sexes. This hypothesis obviously fails to account for the nonrandom occurrence of recombinants in our experiments, unless it is applied to the chromosomes of gonial cells. Friesen's omission of this application apparently indicates abandonment of his own earlier proposal that crossovers induced in males were of spermatogonial origin.

Another possible mode of action of x-rays in inducing crossing over which more nearly conforms to our experimental results has recently been ad-

vanced by Cooper[4] as a result of observations of chiasma-like configurations in Drosophila gonial cells of both sexes. While it is clear that such chiasmata do not regularly lead to spontaneous recombination, at least in the male, Cooper supposes that these configurations may be competent targets for ionizing particles, and he concludes that x-ray induced crossovers are probably of gonial origin. This would be the place of origin of the crossovers found in this experiment in view of the tendency for complementary crossovers to vary in either direction from the expected.

Summary.—The induced crossover offspring from testcrossed *S Pfd/Cy lt*[3] *L*[4] females x-rayed with 2250 r as adults were so distributed that their meiotic origin is unlikely. The effects of the x-rays were evident for as long as 31 days, with a peak in recombination between 4 and 8 days after treatment. Wide variations from female to female were observed in total per cent recombination, in the relative numbers of recombinants from adjacent regions, and in the distribution of complementary crossover classes within each region. Some tendency toward clustering of the recombinants among the testcross offspring was revealed. These results are indicative of some oögonial influence upon recombinants. This influence may be merely a weakening of oögonial chromosomes followed much later by meiotic crossing over at the weak point, or it may be completion of crossing over in the oögonia. The data on the variable balance of complementary classes favors the latter hypothesis.

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